Exhibit G

#41

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Gordon et al.	Examiner:	J. Chambers
Serial No:	07/938,322	Group Art Unit:	1804
Filed:	August 31, 1992	Attorney Docket:	IGI-017

Title: TRANSGENIC ANIMALS SECRETING DESIRED PROTEINS INTO MILK

VIA HAND DELIVERY

Honorable Commissioner of
Patent and Trademarks
Washington, DC 20231

FIRST DECLARATION UNDER 37 CFR 1.132 OF KATHERINE GORDON

1. I hold a Ph.D. degree in Biology from Wesleyan University, and I have worked in the field of molecular biology and gene expression for approximately fifteen (15) years. Currently, I am President and C.E.O. of Apollo Genetics, a biotechnology firm involved working in the field of aging. I was previously employed by Integrated Genetics, Inc. of Framingham, Massachusetts from 1984 to 1989, and then with Genzyme, Inc. from 1989 to 1991 after that company acquired Integrated Genetics, the last position being Associate Director. From the beginning its existence

in 1985, I was responsible for the scientific aspects of the transgenic program at Integrated Genetics, and then at Genzyme after the acquisition of Integrated Genetics.

2. I am the co-inventor of the technology claimed in the above-referenced patent application (attached hereto as Appendix A), and I have carefully studied the patent application. This application discloses methods and gene constructs for producing a recombinant protein which is secreted into the milk of a lactating transgenic animal.

3. I have carefully studied those portions of the U.S. Patent Office Actions dated April 19, 1993 and April 7, 1992, which detail the rejection of the pending claims under 35 U.S.C. §112, first paragraph (at pages 2-4 of the April 19, 1993 Office Action, and pages 2-4 of the April 7, 1992 Office Action, which are attached hereto as Appendices B and C, respectively). I understand that the Examiner has objected to the specification as failing to provide an adequate description of, and enablement for, a method for the production of a recombinant protein in the milk of a transgenic mammal. I also understand that the Examiner has held the specification as not enabling for DNA sequences other than those comprising a whey acid protein promoter, arguing that there is insufficient evidence in the specification to indicate that all milk protein promoters can be used with success for the expression of heterologous polypeptide in a transgenic mammal without undue experimentation. I respectfully disagree. Furthermore, it is my understanding that, during the personal interview of 16 June 1993, the Examiner requested a declaration that essentially demonstrates that a method for making a non-human transgenic mammal was supported by the originally-filed specification. I identify portions of the written description, and information which was within the ordinary skill of the art at the time the claimed invention was made, to illustrate this demonstration.

4. At the time the claimed invention was made, one of ordinary skill in the art could, having used the knowledge available to such a person and the disclosure in the present application,

employed the claimed method to generate a transgenic mammal that (i) expressed a recombinant protein in its mammary epithelia under lactating conditions, and (ii) secreted the recombinant protein into its milk. As set forth at pages 1-2 and 7-9 of the present application, it was generally understood that foreign DNA introduced into an embryo, for example, by microinjection or retroviral infection, could be integrated into the chromosomal DNA of an animal and carried in both germ-line and somatic cells. I illustrate below how, in light of the teachings of the present application, the claimed method could have been successfully carried out by one of ordinary skill in the art without any additional inventive contribution over that originally disclosed in the present application. While the following discussions may, at times, be written in the present tense to promote ease of reading, it should be understood to represent the state of the art at the time the claimed invention was made. Furthermore, the articles cited below are merely exemplary of references and knowledge generally available to one of ordinary skill in the art at the time the claimed invention was made.

5. In the instance of microinjection protocols, transgenic animal production is typically divided into four main phases: (a) preparation of the animals; (b) recovery and maintenance *in vitro* of one or two-celled embryos; (c) microinjection of the embryos' and (d) reimplantation of embryos into pseudo-pregnant females. The methods used for producing transgenic livestock at the time the claimed invention was made, as well as today, differ only in scale and minor detail from those used to produce transgenic mice. Compare, for example, Gordon et al. (1983) *Methods in Enzymology* 101:411, and Gordon et al. (1980) *PNAS* 77:7380 with Hammer et al. (1985) *Nature* 315:680.

6. One step of the preparatory phase comprises synchronizing the estrus cycle of at least the donor females, and inducing superovulation in the donor females prior to mating. Superovulation typically involves administering drugs at an appropriate stage of the estrus cycle to stimulate follicular development, followed by treatment with drugs to synchronize estrus and

initiate ovulation. See Gordon et al. (1980) *PNAS* 77:7380 describing superovulation of mice; Hammer et al. (1985) *Nature* 315:600 describing superovulation of sheep; Wall et al. (1985) *Biol. Reprod.* 32:645 describing superovulation of pigs; and Hasler et al. (1983) *Theriogenology* 19:83 describing superovulatory responses in cows. Superovulation increases the likelihood that a large number of healthy embryos will be available after mating, and further allows the practitioner to control the timing of experiments.

7. Another aspect of animal preparation comprises preparing vasectomized males. After microinjection, embryos must be reimplanted into pseudo-pregnant foster mothers for continued development. Recipient females are prepared by mating mature females with vasectomized males. Because the males are sterile, the endogenously ovulated eggs are not fertilized and cannot compete with the microinjected embryos that are surgically implanted. Vasectomy is a simple procedure and was well known to those skilled in the art at the time the claimed invention was made.

8. After mating, one or two-cell fertilized eggs from the superovulated females are harvested for microinjection. A variety of protocols useful in collecting eggs from various mammals were known to those skilled in the art at the time the claimed invention was made. For example, in one approach, oviducts of fertilized superovulated females can be surgically removed and isolated in a buffer solution/culture medium, and fertilized eggs expressed from the isolated ovaries. See, Gordon et al. (1980) *PNAS* 77:7380; and Gordon et al. (1983) *Methods in Enzymology* 101:411 describing the recovery of mouse embryos. Alternatively, the ovaries can be cannulated and the fertilized eggs can be surgically collected from the ovaries of anesthetized animals by flushing with buffer solution/culture medium, thereby eliminating the need to sacrifice the animal. See Hammer et al. (1985) *Nature* 315:600. The timing of the embryo harvest after mating of the superovulated females can depend on the length of the fertilization process and the time required for adequate enlargement of the pronuclei. This temporal waiting

period can range from, for example, 6 to 9 hours for mice and up to 48 hours for larger mammals. Fertilized eggs appropriate for microinjection, such as one-cell ova containing pronuclei, or two-cell embryos, can be identified under a dissecting microscope.

9. The equipment and reagents needed for microinjection of the isolated embryos of non-murine animals, including livestock, were well known to those skilled in the art at the time the claimed invention was made and are similar to that used for the mouse. See, for example, Gordon et al. (1983) *Methods in Enzymology* 101:411; and Gordon et al. (1980) *PNAS* 77:7380, describing equipment and reagents for microinjecting embryos. Briefly, fertilized eggs are positioned with an egg holder (fabricated from 1 mm glass tubing), which is attached to a micro-manipulator, which is in turn coordinated with a dissecting microscope fitted with differential interference contrast optics. Where visualization of pronuclei is difficult because of optically dense cytoplasmic material, centrifugation of the embryos can be carried out without compromising embryo viability. Wall et al. (1985) *Biol. Reprod.* 32:645. The microneedles used for injection, like the egg holder, can also be pulled from glass tubing. The tip of a microneedle is allowed to fill with plasmid suspension by capillary action. By microscopic visualization, the microneedle is then inserted into the pronucleus of a cell held by the egg holder, and plasmid suspension injected into the pronucleus. If injection is successful, the pronucleus will swell noticeably. The microneedle is then withdrawn, and cells which survive the microinjection (e.g. those which do not lyse) are subsequently used for implantation in a host female.

10. Techniques and equipment for reimplantation of embryos into pseudo-pregnant recipient females were also well known at the time the claimed invention was made. The equipment required for oviductal implantation is typically the same as that needed for vasectomy of the males, except for the additional requirement of an implantation pipette. Microinjected embryos are collected in the implantation pipette, the pipette inserted into the surgically exposed oviduct

of a recipient female, and the microinjected eggs expelled into the oviduct. After withdrawal of the implantation pipette, any surgical incision can be closed, and the embryos allowed to continue gestation in the foster mother. See, for example, Gordon et al. (1983) *Methods in Enzymology* 101:411; and Gordon et al. (1980) *PNAS* 77:7390 describing reimplantation of mouse embryos; Hammer et al. (1985) *Nature* 315:600 describing reimplantation of sheep embryos; and Wall et al. (1985) *Biol. Reprod.* 32:645 describing reimplantation of pig embryos.

11. As I illustrate above, the claimed method could have been successfully carried out by one of ordinary skill in the art without any additional inventive contribution over that originally disclosed in the present application. At the time the invention was made, the methods used for producing transgenic mammals, such as livestock, differed only in scale and minor detail from those used to produce transgenic mice. Indeed, such methods as existed at the time the claimed invention was made continue to be used today to generate other transgenic mammals in addition to transgenic mice.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that such willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Katherine Gordon

Dated: October 4 1993 Signature: